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(21) International Application Number: PCT/US99/23181 (22) International Filing Date: 5 October 1999 (05.10.99) (30) Priority Data: 60/103,050 5 October 1998 (05.10.98) US (71) Applicant: EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US). (72) Inventors: WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US). FAN, Hao; 19712 6th Drive S.E., Bothell, WA 98012 (US). NIGGEMEYER, Jennifer, L.; 21315 2nd Avenue S.E., Bothell, WA 98021 (US). (74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 6 July 2000 (06.07.00)	
(54) Title: HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSEN- SITIVE RESPONSE (57) Abstract The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.		

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HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 60/103,050, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to active fragments of a hypersensitive
10 response elicitor which fragments do not elicit a hypersensitive response.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular
bacterial growth, symptom development, and disease development in the host plant;
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a
particular type of incompatible interaction occurring, without progressive disease
symptoms. During compatible interactions on host plants, bacterial populations
20 increase dramatically and progressive symptoms occur. During incompatible
interactions, bacterial populations do not increase, and progressive symptoms do not
occur.

The hypersensitive response is a rapid, localized necrosis that is
associated with the active defense of plants against many pathogens (Kiraly, Z.,
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily
30 observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

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"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H.,

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et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

"PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

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The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

5

SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in
10 conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling
15 insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects
20 on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control
25 insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

5 Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ.
10 ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

15

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not
20 elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or
25 proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the
30 following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

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An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

5 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

10	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	1	5	10	15
	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	20	25	30	
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	35	40	45	
15	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	50	55	60	
	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	
20	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	85	90	95	
	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	100	105	110	
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	115	120	125	
25	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	130	135	140	
	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	145	150	155	
30	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	165	170	175	
	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	180	185	190	
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	195	200	205	
35	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	210	215	220	
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	225	230	235	
																			240	

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270

5 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
 275 280 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290 295 300

10 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
325 330 335

Asn Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

20

CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCTA CACCGTTACG 60

GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC 120

GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGA ACTCA TGATGCAGAT TCAGCCGGGG 180

25 CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG 240

TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG 300

CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGA ACT GGC GGGAATG 360

ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC 420

CGATCATTAA GATAAAGGCG GCTTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT 480

30 CACCGTCGGC GTCACTCAGT AACAGTATC CATCATGATG CCTACATCGG GATCGGCGTG 540

GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600

AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660

TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCACGCG TGGATAAACT 720

GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT 780

35 GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840

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TTTCCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA 900
 TGC GTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960
 CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC 1020
 CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG 1080
 5 CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140
 GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200
 GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260
 CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320
 TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380
 10 GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440
 CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500
 TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560
 GGCTGTCGTC GGC GATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620
 ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680
 15 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740
 ACGCACATT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCTC 1800
 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860
 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCTTTAG 1920
 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980
 20 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040
 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100
 GTTCGTCATC ATCTTCTCTCC ATCTGGGCGA CCTGATCGGT T 2141

25 The hypersensitive response elicitor polypeptide or protein derived
 from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.
 No. 23 as follows:

30 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
 1 5 10 15
 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
 20 25 30

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Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
 35 40 45
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
 50 55 60
 5 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
 65 70 75 80
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
 85 90 95
 10 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
 100 105 110
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
 115 120 125
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
 130 135 140
 15 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
 145 150 155 160
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175
 20 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
 195 200 205
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
 210 215 220
 25 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
 225 230 235 240
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
 245 250 255
 30 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
 260 265 270
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
 275 280 285
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
 290 295 300
 35 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
 305 310 315 320
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
 325 330 335
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn

340

350

5 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385 390 395 400

Gly Ala Ala

20	AAGCTTCGGC	ATGGCACGTT	TGACCGTTGG	GTCCGCAGGG	TACGTTTGAA	TTATTCCATAA	60
	GAGGAATACG	TTATGAGTCT	GAATACAAGT	GGGCTGGGAG	CGTCAACGAT	GCAAATTTCT	120
	ATCGGCGGTG	CGGGCGGAAA	TAACGGGTTG	CTGGGTACCA	GTCGCCAGAA	TGCTGGGTTG	180
	GGTGGCAATT	CTGCACTGGG	GCTGGGCGGC	GGTAATCAAA	ATGATACCGT	CAATCAGCTG	240
	GCTGGCTTAC	TCACCGGCAT	GATGATGATG	ATGAGCATGA	TGGGCGGTGG	TGGGCTGATG	300
25	GGCGGTGGCT	TAGGCGGTGG	CTTAGGTAAT	GGCTTGGGTG	GCTCAGGTGG	CCTGGGCGAA	360
	GGACTGTCGA	ACGCGCTGAA	CGATATGTTA	GGCGGTTCCG	TGAACACGCT	GGGCTCGAAA	420
	GGCGGCAACA	ATACCACTTC	AACAACAAAT	TCCCCGCTGG	ACCAGGCGCT	GGGTATTAAC	480
	TCAACGTCCC	AAAACGACGA	TTCCACCTCC	GGCACAGATT	CCACCTCAGA	CTCCAGCGAC	540
	CCGATGCAGC	AGCTGCTGAA	GATGTTCAGC	GAGATAATGC	AAAGCCTGTT	TGGTGATGGG	600
30	CAAGATGGCA	CCCAGGGCAG	TTCCTCTGGG	GGCAAGCAGC	CGACCGAAGG	CGAGCAGAAC	660
	GCCTATAAAA	AAGGAGTCAC	TGATGCGCTG	TCGGGCCTGA	TGGGTAATGG	TCTGAGCCAG	720
	CTCCTTGCCA	ACGGGGGACT	GGGAGGTGGT	CAGGGCGGTA	ATGCTGGCAC	GGGTCTTGAC	780
	GGTTCGTGCG	TGGGCGGCAA	AGGGCTGCAA	AACCTGAGCG	GGCCGGTGGA	CTACCAGCAG	840

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TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT 900
 ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960
 GCGAAGGAAA TCGGTCAAGT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC 1020
 CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC 1080
 5 AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140
 ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200
 GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260
 CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

10

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

15 ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG 60
 GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120
 20 CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180
 CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240
 AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300
 25 CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360
 CAGGGCGGCG GGCAGATCGG CGATAATCCT TACTGAAAG CCATGCTGAA GCTTATTGCA 420
 30 CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480
 TCTTCCGGTA CTCTTCATC TGGCGGTTCC CCTTTAACG ATCTATCAGG GGGGAAGGCC 540
 CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCGTCA GTACCTTCTC ACCCCCATCC 600
 35 ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG 660
 GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720
 40 AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780
 GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA 840
 GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CCGTGCCAGC 900
 45 CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CCGTGATGCC 960
 AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020
 50 AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC 1080
 AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140

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TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200
 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260
 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320
 GCCAACCTGA AGGTGGCTGA ATGA 1344

10

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
 1 5 10 15
 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
 20 20 25 30
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
 35 40 45
 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
 25 50 55 60
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
 65 70 75 80
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
 85 90 95
 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
 100 105 110
 35 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
 115 120 125
 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
 40 130 135 140
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
 145 150 155 160
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
 165 170 175
 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
 180 185 190
 50 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
 195 200 205
 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
 55 210 215 220

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	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225					230					235					240	
5	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
				245						250					255		
	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
10	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
			275					280					285				
	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
		290					295					300					
15	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
20				325						330					335		
	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
				340					345					350			
25	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	
			355					360					365				
	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	
		370					375					380					
30	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	
	385				390						395					400	
	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	
35				405						410					415		
	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	
				420					425					430			
40	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu		
			435					440					445				

45 This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

50 Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

- 15 -

	GATGCGCTGG	TTATCGACAA	TCAGCTGGGG	CTGCATCATA	CCAATCCTGA	ACCGCATGAG	2040
	ATTCTTGATA	TGGGGCATT	AGGCAGCCTG	GCGTTACAGG	AGGGCAAGCT	TCACTATTTT	2100
5	GACCAGCTGA	CCAAAGGGTG	GACTGGCGCG	GAGTCAGATT	GTAAGCAGCT	GAAAAAAGGC	2160
	CTGGATGGAG	CAGCTTATCT	ACTGAAAGAC	GGTGAAGTGA	AACGCCTGAA	TATTAATCAG	2220
10	AGCACCTCCT	CTATCAAGCA	CGGAACGGAA	AACGTTTTTT	CGCTGCCGCA	TGTGCGCAAT	2280
	AAACCGGAGC	CGGGAGATGC	CCTGCAAGGG	CTGAATAAAG	ACGATAAGGC	CCAGGCCATG	2340
	GCGGTGATTG	GGGTAAATAA	ATACCTGGCG	CTGACGGAAA	AAGGGGACAT	TCGCTCCTTC	2400
15	CAGATAAAAC	CCGGCACCCA	GCAGTTGGAG	CGGCCGGCAC	AAACTCTCAG	CCGCGAAGGT	2460
	ATCAGCGGCG	AACTGAAAGA	CATTTCATGTC	GACCACAAGC	AGAACCTGTA	TGCCTTGACC	2520
20	CACGAGGGAG	AGGTGTTTCA	TCAGCCGCGT	GAAGCCTGGC	AGAATGGTGC	CGAAAGCAGC	2580
	AGCTGGCACA	AACTGGCGTT	GCCACAGAGT	GAAAGTAAGC	TAAAAAGTCT	GGACATGAGC	2640
	CATGAGCACA	AACCGATTGC	CACCTTTGAA	GACGGTAGCC	AGCATCAGCT	GAAGGCTGGC	2700
25	GGCTGGCACG	CCTATGCGGC	ACCTGAACGC	GGGCCGCTGG	CGGTGGGTAC	CAGCGGTTCA	2760
	CAAACCGTCT	TTAACCGACT	AATGCAGGGG	GTGAAAGGCA	AGGTGATCCC	AGGCAGCGGG	2820
30	TTGACGGTTA	AGCTCTCGGC	TCAGACGGGG	GGAATGACCG	GCGCCGAAGG	GCGCAAGGTC	2880
	AGCAGTAAAT	TTTCCGAAAG	GATCCGCGCC	TATGCGTTCA	ACCCAACAAT	GTCCACGCCG	2940
	CGACCGATTA	AAAATGCTGC	TTATGCCACA	CAGCACGGCT	GGCAGGGGCG	TGAGGGGTTG	3000
35	AAGCCGTTGT	ACGAGATGCA	GGGAGCGCTG	ATTAAACAAC	TGGATGCGCA	TAACGTTTCG	3060
	CATAACGCGC	CACAGCCAGA	TTTGCAGAGC	AAACTGGAAA	CTCTGGATT	AGGCGAACAT	3120
40	GGCGCAGAAT	TGCTTAACGA	CATGAAGCGC	TTCCGCGACG	AACTGGAGCA	GAGTGCAACC	3180
	CGTTCGGTGA	CCGTTTTAGG	TCAACATCAG	GGAGTGCTAA	AAAGCAACGG	TGAAATCAAT	3240
	AGCGAATTTA	AGCCATCGCC	CGGCAAGGCG	TTGGTCCAGA	GCTTTAACGT	CAATCGCTCT	3300
45	GGTCAGGATC	TAAGCAAGTC	ACTGCAACAG	GCAGTACATG	CCACGCCGCC	ATCCGCAGAG	3360
	AGTAAACTGC	AATCCATGCT	GGGGCACTTT	GTCAGTGCCG	GGGTGGATAT	GAGTCATCAG	3420
50	AAGGGCGAGA	TCCCGCTGGG	CCGCCAGCGC	GATCCGAATG	ATAAAACCGC	ACTGACCAAA	3480
	TCGCGTTTTAA	TTTLAGATAC	CGTGACCATC	GGTGAAGTGC	ATGAACTGGC	CGATAAGGCG	3540
	AAACTGGTAT	CTGACCATAA	ACCCGATGCC	GATCAGATAA	AACAGCTGCG	CCAGCAGTTC	3600
55	GATACGCTGC	GTGAAAAGCG	GTATGAGAGC	AATCCGGTGA	AGCATTACAC	CGATATGGGC	3660
	TTCACCCATA	ATAAGGCGCT	GGAAGCAAAC	TATGATGCGG	TCAAAGCCTT	TATCAATGCC	3720
60	TTTAAGAAAG	AGCACCACGG	CGTCAATCTG	ACCACGCGTA	CCGTACTGGA	ATCACAGGGC	3780
	AGTGCGGAGC	TGGCGAAGAA	GCTCAAGAAT	ACGCTGTTGT	CCCTGGACAG	TGGTGAAAGT	3840
65	ATGAGCTTCA	GCCGGTCATA	TGGCGGGGGC	GTCAGCACTG	TCTTTGTGCC	TACCCTTAGC	3900

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	AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG	4020
5	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
	ACCAGTGCAG GTAACGCCAG TGA CTGGTTG AGCGCAAAAC ATAAATCAG CCCGGA CTTG	4140
10	CGTATCGGCG CTGCTGTGAG TGGCACCCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAACTGACG	4320
15	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
	GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAAC	4440
	CTGGCCGCCG GCTCGGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
20	GCCAGCAATA ACCGCCCAAC CTTCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCCGGAT CTTCCCGGCA	4620
25	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
	ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
30	TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA	4860
	GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAACT GGTGATACGT	4920
35	CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC	4980
	AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTGAGC TGCTACACAA ACATTTTCAT	5040
40	GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100
	CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG	5160
	GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT	5220
45	CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC	5280
	GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG	5340
50	AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC	5400
	CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG	5460
	CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGC TGGAATGAA GAGCTAA	5517

55

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

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	Met	Glu	Leu	Lys	Ser	Leu	Gly	Thr	Glu	His	Lys	Ala	Ala	Val	His	Thr	
	1				5					10					15		
5	Ala	Ala	His	Asn	Pro	Val	Gly	His	Gly	Val	Ala	Leu	Gln	Gln	Gly	Ser	
				20					25					30			
	Ser	Ser	Ser	Ser	Pro	Gln	Asn	Ala	Ala	Ala	Ser	Leu	Ala	Ala	Glu	Gly	
				35				40					45				
10	Lys	Asn	Arg	Gly	Lys	Met	Pro	Arg	Ile	His	Gln	Pro	Ser	Thr	Ala	Ala	
		50					55					60					
	Asp	Gly	Ile	Ser	Ala	Ala	His	Gln	Gln	Lys	Lys	Ser	Phe	Ser	Leu	Arg	
15		65				70					75					80	
	Gly	Cys	Leu	Gly	Thr	Lys	Lys	Phe	Ser	Arg	Ser	Ala	Pro	Gln	Gly	Gln	
					85					90					95		
20	Pro	Gly	Thr	Thr	His	Ser	Lys	Gly	Ala	Thr	Leu	Arg	Asp	Leu	Leu	Ala	
				100					105					110			
	Arg	Asp	Asp	Gly	Glu	Thr	Gln	His	Glu	Ala	Ala	Ala	Pro	Asp	Ala	Ala	
			115					120					125				
25	Arg	Leu	Thr	Arg	Ser	Gly	Gly	Val	Lys	Arg	Arg	Asn	Met	Asp	Asp	Met	
		130					135					140					
	Ala	Gly	Arg	Pro	Met	Val	Lys	Gly	Gly	Ser	Gly	Glu	Asp	Lys	Val	Pro	
30		145				150					155					160	
	Thr	Gln	Gln	Lys	Arg	His	Gln	Leu	Asn	Asn	Phe	Gly	Gln	Met	Arg	Gln	
					165					170					175		
35	Thr	Met	Leu	Ser	Lys	Met	Ala	His	Pro	Ala	Ser	Ala	Asn	Ala	Gly	Asp	
				180					185					190			
	Arg	Leu	Gln	His	Ser	Pro	Pro	His	Ile	Pro	Gly	Ser	His	His	Glu	Ile	
			195					200					205				
40	Lys	Glu	Glu	Pro	Val	Gly	Ser	Thr	Ser	Lys	Ala	Thr	Thr	Ala	His	Ala	
		210					215					220					
	Asp	Arg	Val	Glu	Ile	Ala	Gln	Glu	Asp	Asp	Asp	Ser	Glu	Phe	Gln	Gln	
45		225				230					235					240	
	Leu	His	Gln	Gln	Arg	Leu	Ala	Arg	Glu	Arg	Glu	Asn	Pro	Pro	Gln	Pro	
					245					250					255		
50	Pro	Lys	Leu	Gly	Val	Ala	Thr	Pro	Ile	Ser	Ala	Arg	Phe	Gln	Pro	Lys	
				260					265					270			
	Leu	Thr	Ala	Val	Ala	Glu	Ser	Val	Leu	Glu	Gly	Thr	Asp	Thr	Thr	Gln	
				275				280					285				
55	Ser	Pro	Leu	Lys	Pro	Gln	Ser	Met	Leu	Lys	Gly	Ser	Gly	Ala	Gly	Val	
		290					295					300					
	Thr	Pro	Leu	Ala	Val	Thr	Leu	Asp	Lys	Gly	Lys	Leu	Gln	Leu	Ala	Pro	
60		305				310					315					320	
	Asp	Asn	Pro	Pro	Ala	Leu	Asn	Thr	Leu	Leu	Lys	Gln	Thr	Leu	Gly	Lys	
					325					330					335		
65	Asp	Thr	Gln	His	Tyr	Leu	Ala	His	His	Ala	Ser	Ser	Asp	Gly	Ser	Gln	
				340					345					350			

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His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
 355 360 365
 5 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
 370 375 380
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
 385 390 395 400
 10 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
 405 410 415
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
 420 425 430
 15 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
 435 440 445
 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
 450 455 460
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
 465 470 475 480
 25 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
 485 490 495
 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
 500 505 510
 30 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
 515 520 525
 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
 530 535 540
 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
 545 550 555 560
 40 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
 565 570 575
 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
 580 585 590
 45 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
 595 600 605
 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
 610 615 620
 50 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
 625 630 635 640
 55 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
 645 650 655
 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
 660 665 670
 60 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
 675 680 685

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	Ser	Leu	Ala	Leu	Gln	Glu	Gly	Lys	Leu	His	Tyr	Phe	Asp	Gln	Leu	Thr	
	690						695					700					
5	Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly	
	705					710					715					720	
	Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu	
					725					730					735		
10	Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val	
				740					745						750		
	Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu	
			755					760					765				
15	Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly	
		770					775					780					
	Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe	
20		785				790					795					800	
	Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu	
					805					810					815		
25	Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His	
				820					825						830		
	Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln	
30			835					840					845				
	Pro	Arg	Glu	Ala	Trp	Gln	Asn	Gly	Ala	Glu	Ser	Ser	Ser	Trp	His	Lys	
		850					855					860					
35	Leu	Ala	Leu	Pro	Gln	Ser	Glu	Ser	Lys	Leu	Lys	Ser	Leu	Asp	Met	Ser	
		865				870					875					880	
	His	Glu	His	Lys	Pro	Ile	Ala	Thr	Phe	Glu	Asp	Gly	Ser	Gln	His	Gln	
					885					890					895		
40	Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro	
				900					905						910		
	Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met	
45			915					920					925				
	Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys	
		930					935					940					
50	Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val	
		945				950					955					960	
	Ser	Ser	Lys	Phe	Ser	Glu	Arg	Ile	Arg	Ala	Tyr	Ala	Phe	Asn	Pro	Thr	
					965					970					975		
55	Met	Ser	Thr	Pro	Arg	Pro	Ile	Lys	Asn	Ala	Ala	Tyr	Ala	Thr	Gln	His	
				980					985						990		
	Gly	Trp	Gln	Gly	Arg	Glu	Gly	Leu	Lys	Pro	Leu	Tyr	Glu	Met	Gln	Gly	
			995					1000					1005				
60	Ala	Leu	Ile	Lys	Gln	Leu	Asp	Ala	His	Asn	Val	Arg	His	Asn	Ala	Pro	
		1010					1015					1020					
65	Gln	Pro	Asp	Leu	Gln	Ser	Lys	Leu	Glu	Thr	Leu	Asp	Leu	Gly	Glu	His	
		1025				1030					1035					1040	

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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
 1045 1050 1055
 5 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
 1060 1065 1070
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085
 10 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
 1090 1095 1100
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
 1105 1110 1115 1120
 15 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135
 20 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165
 25 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
 1185 1190 1195 1200
 30 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
 1205 1210 1215
 35 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
 1220 1225 1230
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
 1235 1240 1245
 40 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
 1250 1255 1260
 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
 1265 1270 1275 1280
 45 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
 1285 1290 1295
 50 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
 1300 1305 1310
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
 1315 1320 1325
 55 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
 1330 1335 1340
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
 1345 1350 1355 1360
 60 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
 1365 1370 1375

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	Ser	Pro	Asp	Leu	Arg	Ile	Gly	Ala	Ala	Val	Ser	Gly	Thr	Leu	Gln	Gly	
				1380					1385						1390		
5	Thr	Leu	Gln	Asn	Ser	Leu	Lys	Phe	Lys	Leu	Thr	Glu	Asp	Glu	Leu	Pro	
			1395					1400					1405				
	Gly	Phe	Ile	His	Gly	Leu	Thr	His	Gly	Thr	Leu	Thr	Pro	Ala	Glu	Leu	
		1410				1415					1420						
10	Leu	Gln	Lys	Gly	Ile	Glu	His	Gln	Met	Lys	Gln	Gly	Ser	Lys	Leu	Thr	
	1425					1430					1435					1440	
	Phe	Ser	Val	Asp	Thr	Ser	Ala	Asn	Leu	Asp	Leu	Arg	Ala	Gly	Ile	Asn	
15					1445					1450					1455		
	Leu	Asn	Glu	Asp	Gly	Ser	Lys	Pro	Asn	Gly	Val	Thr	Ala	Arg	Val	Ser	
			1460						1465					1470			
20	Ala	Gly	Leu	Ser	Ala	Ser	Ala	Asn	Leu	Ala	Ala	Gly	Ser	Arg	Glu	Arg	
		1475						1480					1485				
	Ser	Thr	Thr	Ser	Gly	Gln	Phe	Gly	Ser	Thr	Thr	Ser	Ala	Ser	Asn	Asn	
		1490				1495						1500					
25	Arg	Pro	Thr	Phe	Leu	Asn	Gly	Val	Gly	Ala	Gly	Ala	Asn	Leu	Thr	Ala	
	1505					1510					1515					1520	
	Ala	Leu	Gly	Val	Ala	His	Ser	Ser	Thr	His	Glu	Gly	Lys	Pro	Val	Gly	
30					1525					1530					1535		
	Ile	Phe	Pro	Ala	Phe	Thr	Ser	Thr	Asn	Val	Ser	Ala	Ala	Leu	Ala	Leu	
			1540						1545					1550			
35	Asp	Asn	Arg	Thr	Ser	Gln	Ser	Ile	Ser	Leu	Glu	Leu	Lys	Arg	Ala	Glu	
		1555						1560					1565				
	Pro	Val	Thr	Ser	Asn	Asp	Ile	Ser	Glu	Leu	Thr	Ser	Thr	Leu	Gly	Lys	
		1570				1575						1580					
40	His	Phe	Lys	Asp	Ser	Ala	Thr	Thr	Lys	Met	Leu	Ala	Ala	Leu	Lys	Glu	
	1585					1590					1595					1600	
	Leu	Asp	Asp	Ala	Lys	Pro	Ala	Glu	Gln	Leu	His	Ile	Leu	Gln	Gln	His	
45					1605					1610					1615		
	Phe	Ser	Ala	Lys	Asp	Val	Val	Gly	Asp	Glu	Arg	Tyr	Glu	Ala	Val	Arg	
			1620						1625					1630			
50	Asn	Leu	Lys	Lys	Leu	Val	Ile	Arg	Gln	Gln	Ala	Ala	Asp	Ser	His	Ser	
		1635						1640					1645				
	Met	Glu	Leu	Gly	Ser	Ala	Ser	His	Ser	Thr	Thr	Tyr	Asn	Asn	Leu	Ser	
		1650					1655					1660					
55	Arg	Ile	Asn	Asn	Asp	Gly	Ile	Val	Glu	Leu	Leu	His	Lys	His	Phe	Asp	
	1665					1670					1675					1680	
	Ala	Ala	Leu	Pro	Ala	Ser	Ser	Ala	Lys	Arg	Leu	Gly	Glu	Met	Met	Asn	
60					1685					1690					1695		
	Asn	Asp	Pro	Ala	Leu	Lys	Asp	Ile	Ile	Lys	Gln	Leu	Gln	Ser	Thr	Pro	
			1700						1705					1710			
65	Phe	Ser	Ser	Ala	Ser	Val	Ser	Met	Glu	Leu	Lys	Asp	Gly	Leu	Arg	Glu	
		1715						1720					1725				

	Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val	1730	1735	1740
5	Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser	1745	1750	1755 1760
	Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu		1765	1770 1775
10	Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile		1780	1785 1790
	Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg		1795	1800 1805
15	Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser		1810	1815 1820
	Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser		1825	1830 1835
20				

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

25 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

	ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTA	60
30	ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG	120
	GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG	180
35	GCTGACCCAC AAACCTCAAT AACCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG	240
	GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT	300
	CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC	360
40	GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA	420

This is known as the *dspF* gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

	Met	Thr	Ser	Ser	Gln	Gln	Arg	Val	Glu	Arg	Phe	Leu	Gln	Tyr	Phe	Ser
	1				5					10						15
50	Ala	Gly	Cys	Lys	Thr	Pro	Ile	His	Leu	Lys	Asp	Gly	Val	Cys	Ala	Leu
				20					25					30		
	Tyr	Asn	Glu	Gln	Asp	Glu	Glu	Ala	Ala	Val	Leu	Glu	Val	Pro	Gln	His
			35					40					45			
55	Ser	Asp	Ser	Leu	Leu	Leu	His	Cys	Arg	Ile	Ile	Glu	Ala	Asp	Pro	Gln
							55					60				

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Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80
 5 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110
 10 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125
 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
 130 135
 15

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

20 No. 31 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1 5 10 15
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
 20 25 30
 25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60
 30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
 65 70 75 80
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85 90 95
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
 100 105 110
 35 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
 130 135 140
 40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
 145 150 155 160
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
 165 170 175
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
 180 185 190

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Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
 195 200 205
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
 210 215 220
 5 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
 225 230 235 240
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
 245 250 255
 10 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
 260 265 270
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
 275 280 285
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
 290 295 300
 15 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335
 20 Asn Gln Ala Ala Ala
 340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

25 Further information about the hypersensitive response elicitor derived from
Pseudomonas syringae is found in He, S. Y., H. C. Huang, and A. Collmer,
 "Pseudomonas syringae pv. syringae Harpin_{PS}: a Protein that is Secreted via the Hrp
 Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266
 (1993), which is hereby incorporated by reference. The DNA molecule encoding the
 30 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide
 sequence corresponding to SEQ. ID. No. 32 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTCTG 60
 GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTCT 120
 35 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180
 AAAGTGTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTCT 240
 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

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GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360
 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420
 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480
 AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540
 5 GAAACGGCTG CGTTCCGTTC GGCACGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600
 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660
 AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720
 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780
 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAC CGGTACGTCG 840
 10 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900
 GGGCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960
 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020
 GCCTGA 1026

15 Another potentially suitable hypersensitive response elicitor from
Pseudomonas syringae is disclosed in U.S. Patent Application Serial No. 09/120,817,
 which is hereby incorporated by reference. The protein has a nucleotide sequence of
 SEQ. ID. No. 33 as follows:

20 TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60
 CTGAGTGCGC AGATTTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAGG 120
 CCTCTGAGTG CCGTGCGGAG CAATACCAGT CTTCTGCTG GCGTGTGCAC ACTGAGTCGC 180
 25 AGGCATAGGC ATTTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAGGA ACTTTTAAAA 240
 ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300
 30 AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360
 TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCAGCAT 420
 GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCAG CCACTCGATT TTTCGGCGCT 480
 35 AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA 540
 CCCGAGTGCA CTGTTGTTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCGGA 600
 40 CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660
 TAAATTGATC AGTGATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720
 GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT 780

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CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG 840
 CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900
 5 TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960
 CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020
 10 CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC 1080
 CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140
 CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200
 15 GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA 1260
 CGAGGTTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320
 20 GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380
 CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440
 CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAGGCC GACGATTTCTG GCACGATGGT 1500
 25 TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560
 TAACCACGGC AAGTTCGCCC TGGTGAAGG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620
 30 CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

35 This DNA molecule is known as the *dspE* gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
 1 5 10 15
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
 20 25 30
 45 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
 35 40 45
 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
 50 55 60
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
 65 70 75 80
 55 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
 85 90 95

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	Asn	Ser	Asn	Lys	Lys	Gln	Asp	Thr	Asn	Gln	Glu	Gln	Pro	Asp	Ser	Gln	
				100					105					110			
5	Ala	Pro	Phe	Gln	Asn	Asn	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ala	Asp	Ser	
			115				120						125				
	Gly	Gly	Gly	Gly	Thr	Pro	Asp	Ala	Thr	Gly	Gly	Gly	Gly	Gly	Asp	Thr	
			130				135						140				
10	Pro	Ser	Ala	Thr	Gly	Gly	Gly	Gly	Asp	Thr	Pro	Thr	Ala	Thr	Gly		
	145				150				155						160		
	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly	
				165					170						175		
15	Ser	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly	Glu	Gly	Gly	Val	Thr	
				180					185						190		
20	Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr	
			195					200					205				
	Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile	
		210					215					220					
25	Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp	
	225				230						235					240	
	Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp	
				245					250						255		
30	Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr	
			260						265					270			
35	Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val	
		275					280						285				
	Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln	
		290				295					300						
40	Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala	
	305				310						315					320	
	Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp	
				325					330						335		
45	Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe	
			340					345						350			
50	Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln	
		355						360					365				
	Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly	
		370					375					380					
55	Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr	
	385				390					395						400	
	Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln	
				405						410						415	

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Ala Ser Thr Gln His Thr Glu Leu
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

	Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln	
	1				5					10					15		
15	Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser	
				20					25					30			
	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile	
			35					40					45				
	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly	
		50					55					60					
20	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	
	65					70					75					80	
	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	
					85					90					95		
25	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
30	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155					160	
	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
					165					170					175		
	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
35				180				185						190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
		195						200					205				
	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
		210					215					220					

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	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp
	225					230					235					240
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn
					245					250					255	
5	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln
				260					265					270		
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly
			275					280					285			
10	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser
		290					295					300				
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val
	305					310					315					320
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln
					325					330					335	
15	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met								
						340										

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.
ID. No. 36 as follows:

	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
20	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
	GAGAAGGACA	TCCTCAACAT	CATCGCAGCC	CTCGTGCAGA	AGGCCGCACA	GTCCGGCGGGC	180
	GGCAACACCG	GTAACACCGG	CAACGCGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
25	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCGGCGG	CAATGACAAG	420
	GGCAACGGCG	TGGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCGGCGGCGG	TGCTGGCGCC	540
	GGCGGCGCGG	GTGGCGGTGT	CGGCGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
30	GGCCCGCAGA	ACGCAGGCGA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
	CAGGGCGGCC	TCACCGGCGT	GCTGCAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCCT	CGGCGGCGGC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	840
	GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGGCGCGA	ACCAGCCCGG	TTCGGCGGAT	900

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GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020
 ACGCAGCCGA TGTA 1035

5

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

10

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
 20 25

20

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

25

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
 1 5 10 15
 Leu Leu Ala Met
 20

35

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not
5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from
10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and
15 Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A
20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by
30 growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

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supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

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sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor.

This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

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appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

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Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation). Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

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Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

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Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

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The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

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pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

- 5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

- With regard to the use of the fragments of the hypersensitive response
- 10 elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds
- 15 germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed
- 20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

- Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present
- 25 invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from
- 30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

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The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding
5 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect
10 pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots,
15 propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of
20 the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with
25 the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or
30 polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

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The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5 A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains
10 greater than 500 nM of the fragment.

~~Although not required, this composition may contain additional~~
additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby
30 incorporated by reference.

 Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

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transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

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root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

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After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves
5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using
10 conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the
15 polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including
20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a
25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

30 Example 1 - Bacterial Strains and Plasmids

Escherichia coli strains used in the following examples include DH5 α and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

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(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 α /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then
5 purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)₆ vector pET28(b) which contained a Kan^r gene for selection of transformants.

Example 2 - DNA Manipulation

10 Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR SupermixTM were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR
15 Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual,
20 Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

Example 3 - Fragmentation of *hrpN* Gene

25 A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA
30 and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmpTM 9700 (Perkin-Elmer, Foster City, CA). 45 μ l of SupermixTM (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

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H₂O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA). Amplified DNA was purified with the QIAquick PCR purification kit, digested with

5 Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with

10 the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9

15 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden,

20 Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control,

25 and a clone with only the pET28(b) vector was generated as a negative control.

Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins

Escherichia coli BL21(DE3) strains containing the hrpN clones were

30 grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

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37°C to an OD₆₂₀ of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD₆₂₀ of ca. 0.3 or 0.6-0.8. One millimolar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH₂PO₄, and 0.72 K₂HPO₄), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

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suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated (His)₆-
5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na₂HPO₄, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).
10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

Example 6 - Induction of HR in Tobacco

A 1.5 ml aliquot from the 50 ml cultures grown for small scale
15 purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf
20 panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins
25 for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays

30

Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

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mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)₆-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

Example 9 – Induction of Growth Enhancement (GE)

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked
5 overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium
phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots
and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato
plants per pot were measured and compared with the heights of the control plants
treated only with phosphate buffer. Analyses were done on the heights to determine if
10 there was a significant difference in the height of the plants treated with the truncated
proteins compared with the buffer control, and thereby determine whether the proteins
induced growth enhancement.

Example 10 – Induction of Systemic Acquired Resistance (SAR)

15 Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves
(ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was
covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml
solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five
20 to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and
just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of
TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top
surface of the leaves. The TMV entered the plants through tiny lesions made by the
diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions
25 was counted on both leaves compared with the number of lesions on the negative
control buffer treated leaves. Analyses were done to determine the efficacy of
reducing the number of TMV lesions by the protein fragments compared to the buffer
control. Percentage of efficacy was calculated as: Reduction in TMV lesions (%
efficacy) = 100 x (1 – mean # of lesions on treated leaves/mean # of lesions on buffer
30 control leaves).

Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

5

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

10

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel

15

electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

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the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

5

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large sale purification)

10

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200µg/ml	-
8	1-75	50µg/ml	-
9	1-104	50µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10µg/ml
17	105-168	250µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

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negative control contained several background proteins as expected, but no obviously induced dominant protein.

Example 12 - Induction of HR in Tobacco

5

The full length positive control protein elicited HR down to only 5-7 µg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

10

One definitive domain encompassing a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

20

25

Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

30

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The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

20 **Example 13 – Induction of Plant Growth Enhancement (PGE)**

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 µg/ml, but not at the typical 20 µg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

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Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14%
6	267-403	21%	16% @ 40µg/ml 21% 23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60µg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

Example 14 – Induction of Systemic Acquired Resistance (SAR)

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

Example 15 – Relationship Between HR, PGE, and SAR

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

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fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5 α , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD₆₂₀ of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty μ l of antifoam and 40 μ l of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen μ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

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Table 7

HR test results of HrpZ fragments

<u>HrpZ Fragment</u>	<u>Dilution of Fragment Preparation^a</u>			
	<u>1:2</u>	<u>1:5</u>	<u>1:25</u>	<u>1:125</u>
152-294	+,+ ^b	+,+	+,+	-, -
190-294	-,-	-,-	-,-	-,-

^a The preparations were diluted with MilliQ water.^b The results are indicated for each of two plants. +, HR; -, no HR.

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

<u>HrpZ Fragment</u>	<u>HR elicitation^a</u>	<u>TMV (% efficacy)^b</u>	<u>PGE(% > buffer ht)^c</u>
152-294	+	54.64	4.64
190-294	-	85.25	2.62

^a +, elicits HR in tobacco leaves; -, no HR in tobacco leaves.^b % reduction in TMV lesions in unsprayed leaf of tobacco.^c % greater height than buffer sprayed plants.

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

Exempl 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth

Parsley leaves develop a typical resistance reaction against the soybean
5 pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense
related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa
glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora
megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora
megasperma* f.sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and
10 Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby
incorporated by reference). Then, an oligopeptide of 13 amino acid was identified
within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar
biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to
elicit a complex defense response in parsley cells including H⁺/Ca²⁺ influxes, K⁺/Cl⁻
15 effluxes, active oxygen production, SAR gene induction, and phytoalexin compound
accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide
Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell
78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein
20 also enhanced plant growth, 20 mg of the oligopeptide was synthesized from
Biosynthesis Corp. The synthesized sequence of the peptide is NH₂-Val-Trp-Asn-
Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The
synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer
and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds
25 (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The
soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the
buffer without the peptide were used as a control. After seedlings emerged and the
first two true leaves fully expanded, the height of the tomato seedlings was recorded.
The peptide was not able to elicit the HR in tobacco and other tested plants.
30 However, it had a profound effect on plant growth promotion. Table 9 shows that
tomato seedlings treated with the peptide increased 12.6 % in height, indicating that
the fungal peptide derived from the 42 kDa glycoprotein can promote tomato
seedling growth. Extended studies showed that the peptide also had similar growth

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effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

5

Treatment	Height of seedlings (cm)					Average (cm) % Change	
Buffer	6.0	6.0	6.0	5.5	5.5	5.55	-
	5.5	5.5	5.0	5.0	5.5		
Peptide Solution (100ng/ml)	6.5	6.0	6.5	6.5	6.5	6.25	12.6
	6.0	6.0	6.0	6.0	6.5		

10

15

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.
5
2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.
10
3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.
15
4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.
20
5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.
25
6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.
7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.
30

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8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

9. An isolated fragment according to claim 8, wherein the
5 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10. An isolated DNA molecule encoding a fragment according to claim 1.

10 11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.

12. An isolated DNA molecule according to claim 11, wherein the
15 hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino
20 acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

14. An isolated DNA molecule according to claim 12, wherein the
25 fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

15. An isolated DNA molecule according to claim 12, wherein the
30 fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

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16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

5

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10

19. An expression system transformed with a DNA molecule according to claim 10.

15

20. An expression system according to claim 19, wherein said DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to claim 10.

20

22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

23. A host cell according to claim 21, wherein the DNA molecule is transformed with an expression system.

25

24. A transgenic plant transformed with the DNA molecule of claim 10.

30

25. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

- 64 -

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

15

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

30. A method of imparting disease resistance to plants comprising: applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

30

31. A method according to claim 30, wherein plants are treated during said applying.

- 65 -

32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

5 planting the seeds treated with the fragment of the
hypersensitive response elicitor in natural or artificial soil and
propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:
applying a fragment of a hypersensitive response elicitor
10 protein or polypeptide, which fragment does not elicit a hypersensitive response, in a
non-infectious form to a plant or plant seed under conditions effective to enhance
plant growth.

34. A method according to claim 33, wherein plants are treated
15 during said applying.

35. A method according to claim 33, wherein plant seeds are
treated during said applying, said method further comprising:
planting the seeds treated with the fragment of the
20 hypersensitive response elicitor in natural or artificial soil and
propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:
applying a fragment of a hypersensitive response elicitor protein or
25 polypeptide, which fragment does not elicit a hypersensitive response, in a non-
infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated
during said applying.

30

38. A method according to claim 36, wherein plant seeds are
treated during said applying, said method further comprising:

- 66 -

planting the seeds treated with the fragment of the
hypersensitive response elicitor in natural or artificial soil and
propagating plants from the seeds planted in the soil.

5 39. A method of imparting disease resistance to plants comprising:
 providing a transgenic plant or plant seed transformed with a
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein
or polypeptide, which fragment does not elicit a hypersensitive response, and
 growing the transgenic plant or transgenic plants produced
10 from the transgenic plant seeds under conditions effective to impart disease resistance.

 40. A method according to claim 39, wherein a transgenic plant is
provided.

15 41. A method according to claim 39, wherein a transgenic plant
seed is provided.

 42. A method of enhancing plant growth comprising:
 providing a transgenic plant or a plant seed transformed with a
20 DNA molecule which encodes a fragment of a hypersensitive response elicitor protein
or polypeptide, which fragment does not elicit a hypersensitive response, and
 growing the transgenic plant or transgenic plants produced
from the transgenic plant seeds under conditions effective to enhance plant growth.

25 43. A method according to claim 42, wherein a transgenic plant is
provided.

 44. A method according to claim 42, wherein a transgenic plant
seed is provided.

30 45. A method of insect control for plants comprising:

- 67 -

providing a transgenic plant or plant seed transformed with a
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein
or polypeptide, which fragment does not elicit a hypersensitive response, and
growing the transgenic plant or transgenic plants produced
5 from the transgenic plant seeds under conditions effective to control insects.

46. A method according to claim 45, wherein a transgenic plant is
provided.

10 47. A method according to claim 45, wherein a transgenic plant
seed is provided.

1/2

#1	HARPIN	
	1	403
#3	C-TERMINAL FRAGMENTS	
#4	105	403
#5	169	403
#6	210	403
#7	267	403
	343	403
#8	N-TERMINAL FRAGMENTS	
#9	1	75
#10	1	104
#11	1	168
#12	1	266
	1	342
#13	INTERNAL FRAGMENTS	
#14	76	209
#15	76	168
#16	105	209
#17	169	209
	105	168
#18	SYNTHESIZED OLIGOPEPTIDES	
#19	99	209
#20	137	204
#21	137	180
#22	105	180
#23	150	209
	150	180
	150	179
	137	166
	121	150
	137	156

HARPIN FRAGMENTS DERIVED FROM HrpN OF ERWINIA AMYLOVORA

FIG. 1**SUBSTITUTE SHEET (RULE 26)**

2/2

N1; 5'-GGGAATTCATATGAGTCTGAATACAAGTGGG-3'
N76; 5'-GGGAATTCATATGGGCGGTGGCTTAGGCGGT-3'
N99; 5'-GGCATATGTCTGAACGCGCTGAACGATATG-3'
N105; 5'-GGGAATTCATATGTTAGGCGGTTCGCTGAAC-3'
N110; 5'-GGCATATGCTGAACACGCTGGGCTCGAAA-3'
N137; 5'-GGCATATGTCAACGTCCCAAACGACGAT-3'
N150; 5'-GGCATATGTCCACCTCAGACTCCAGCG-3'
N169; 5'-GGGAATTCATATGCAAAGCCTGTTTGGTGATGGG-3'
N210; 5'-GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'
N267; 5'-GGGAATTCATATGAAAGCGGGCATTCAGGCG-3'
N343; 5'-GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'
C75; 5'-GCAAGCTTAAACAGCCCACCACCGCCCATCAT-3'
C104; 5'-GCAAGCTTAAATCGTTCAGCGCGTTCGACAG-3'
C168; 5'-GCAAGCTTAATATCTCGCTGAACATCTTCAGCAG-3'
C180; 5'-GCAAGCTTAAGGTGCCATCTTGCCCATCAC-3'
C204; 5'-GCAAGCTTAAATCAGTGACTCCTTTTTTATAGGC-3'
C209; 5'-GCAAGCTTAAACAGGCCCGACAGCGCATCAGT-3'
C266; 5'-GCAAGCTTAAACCGATACCGGTACCCACGGC-3'
C342; 5'-GCAAGCTTAATCCGTCGTCATCTGGCTTGCTCAG-3'
C403; 5'-GCAAGCTTAAGCCGCGCCCAGCTTG-3'

OLIGONUCLEOTIDE PRIMERS USED FOR THE CONSTRUCTION
OF THE SUBCLONES OF ERWINIA AMYLOVORA HrpN

FIG. 2

SEQUENCE LISTING

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ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

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<151> 1998-10-05

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<170> PatentIn ver. 2.0

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1

5

10

15

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20

25

30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr

35	40	45
Ser Ala L u Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu		
50	55	60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser		
65	70	75
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys		
85	90	95
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp		
100	105	110
Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln		
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Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp		
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Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
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Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
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Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
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<210> 25

<211> 1344

<212> DNA

<213> *Erwinia amylovora*

<400> 25

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<210> 26

<211> 447

<212> PRT

<213> *Erwinia amylovora*

<400> 26

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Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
 35 40 45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
 50 55 60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
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Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
 85 90 95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
 100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
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Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
 145 150 155 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
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Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
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Val S r Thr Phe Ser Pro Pro Ser Thr Pr Thr Ser Pro Thr Ser Pr
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 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
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 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
 225 230 235 240
 Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
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 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
 260 265 270
 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
 275 280 285
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 290 295 300
 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
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 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
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<210> 27

<211> 5517

<212> DNA

<213> *Erwinia amylovora*

<400> 27

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<210> 28

<211> 1838

<212> PRT

<213> *Erwinia amylovora*

<400> 28

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Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
 35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
 85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
 115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
 145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
 165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
 180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile

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225	230	235 240
Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro		
	245	250 255
Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys		
	260	265 270
Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln		
	275	280 285
Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val		
	290	295 300
Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro		
305	310	315 320
Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys		
	325	330 335
Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln		
	340	345 350
His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr		
	355	360 365
Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys		
	370	375 380
Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys		
385	390	395 400
Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr		
	405	410 415
Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile		
	420	425 430
Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg		
	435	440 445
Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu L u Gly Val Trp		

450 455 460
 Gln Ser Ala Asp Lys Asp Thr His S r Gln Leu Ser Arg Gln Ala Asp
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 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
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 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
 515 520 525
 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
 530 535 540
 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
 545 550 555 560
 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
 565 570 575
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 580 585 590
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 595 600 605
 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
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 625 630 635 640
 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
 645 650 655
 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
 660 665 670
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
 675 680 685
 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
 690 695 700
 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly

705	710	715	720
Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu			
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Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val			
740		745	750
Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu			
755		760	765
Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly			
770		775	780
Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe			
785		790	795
Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu			
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Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His			
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Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln			
	835	840	845
Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys			
	850	855	860
Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser			
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His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln			
	885	890	895
Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro			
	900	905	910
Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met			
	915	920	925
Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys			
	930	935	940
Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val			
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Ser S r Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			

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995		1000		1005	
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Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His					
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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu					
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Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val					
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Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly					
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Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu					
	1090		1095		1100
Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu					
	1105		1110		1115
Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp					
	1125		1130		1135
Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro					
	1140		1145		1150
Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val					
	1155		1160		1165
Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser					
	1170		1175		1180
Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe					
	1185		1190		1195
Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr					
	1205		1210		1215
Thr Asp Met Gly Phe Thr His Asn Lys Ala L u Glu Ala Asn Tyr Asp					

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1235	1240	1245
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Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser		
1265	1270	1275 1280
Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val		
1285	1290	1295
Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly		
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Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly		
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Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile		
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1345	1350	1355 1360
Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile		
1365	1370	1375
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly		
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Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro		
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Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu		
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1445	1450	1455
Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser		
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1475	1480	1485
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Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
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Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
 1825 1830 1835

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 <211> 420
 <212> DNA
 <213> *Erwinia amylovora*

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 <213> *Erwinia amylovora*

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 Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
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 85 90 95
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
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<210> 31

<211> 341

<212> PRT

<213> *Pseudomonas syringae*

<400> 31

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 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60
 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
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 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
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 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
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Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
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Thr Lys Gln Asp Gly Gly Thr Ser Ph Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
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Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

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195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
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260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
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Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
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Asn Gln Ala Ala Ala
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<210> 32

<211> 1026

<212> DNA

<213> *Pseudomonas syringae*

<400> 32

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<210> 33

<211> 1729

<212> DNA

<213> *Pseudomonas syringae*

<400> 33

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<210> 34

<211> 424

<212> PRT

<213> *Pseudomonas syringae*

<400> 34

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 35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
 50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
 65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
 85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
 100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
 115 120 125

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 130 135 140

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 145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly

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Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile 210	215	220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp 225	230	235
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Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr 260	265	270
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Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln 290	295	300
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Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln 355	360	365
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<210> 35

<211> 344

<212> PRT

<213> *Pseudomonas solanacearum*

<220>

<223> Description of Unknown Organism: *Pseudomonas solanacearum*

<400> 35

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Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
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 65 70 75 80

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Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
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Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
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 180 185 190

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 195 200 205

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 225 230 235 240

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 275 280 285

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<210> 36

<211> 1035

<212> DNA

<213> Pseudomonas solanacearum

<400> 36

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<210> 37

<211> 26

<212> PRT

<213> *Xanthomonas campestris* pv. *glycines*

<400> 37

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<210> 38

<211> 20

<212> PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

<400> 38

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<210> 39

<211> 13

<212> PRT

<213> *Phytophthora megasperma*

<400> 39

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INTERNATIONAL SEARCH REPORT

Ints Application No
PCT/US 99/23181

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/195 C12N15/31 C12N1/21 C12N5/10 A01H5/00
A01H5/10 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NÜRNBERGER T, ET AL. : "High Affinity Binding of a Fungal Oligopeptide Elicitor to arslay Plasma Membranes Triggers Multiple Defense Responses" CELL, vol. 78, no. 3, 12 August 1994 (1994-08-12), pages 449-460, XP000882736 Cambridge, Mass. cited in the application the whole document	1,2,10, 11, 19-23, 30-32, 36-38
A	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) the whole document	
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

6 March 2000

Date of mailing of the international search report

03/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax (+31-70) 340-3016

Authorized officer

Bilang, J

Inte. Application No
PCT/US 99/23181

¹ Application No

PCT/US 99/23181

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) the whole document</p>	
A	<p>WEI Z-M, ET AL.: "Harpin, an HR elicitor, activates both defense and growth systems in many commercially important crops" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S96 XP000882741 abstract</p>	
A	<p>NIGGEMEYER J, ET AL.: "Characterization of the functional domains of harpin" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S67 XP000882740 abstract</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/US 99/23181

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
W0 9832844	A	30-07-1998	AU	6043198 A	18-08-1998
W0 9824297	A	11-06-1998	AU	5693598 A	29-06-1998
			EP	0957672 A	24-11-1999

